Multiscale Modeling of Wound Healing

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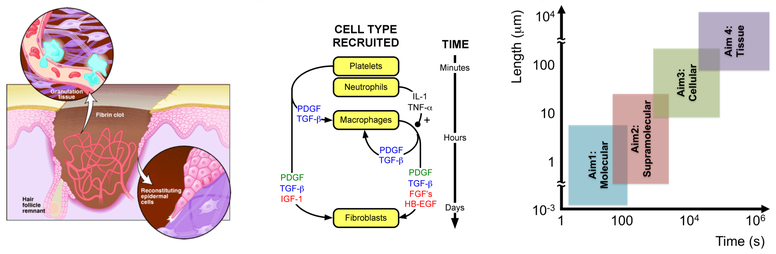
Wound healing is a multiscale phenomenon



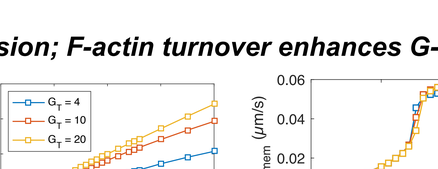
Overall modeling approach & current challenges

PDGF gradient → Receptor activation, DAG patterns → Myosin RLC pattern → Fibroblast morphology, translocation → Fibroblast invasion *in vivo*

Reaction-diffusion (PDEs)	Physico-chemical (stochastic)	Generalized random walks (stochastic)	Multi-agent (stochastic/hybrid)

Molecules to motility problem: how do we connect intracellular dynamics to the mechanics of leading-edge protrusion?

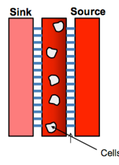
Diversity of cues problem: PDGF is only one spatial cue for fibroblast migration; it is paramount to consider the confluence of chemotaxis, haptotaxis, and durotaxis.



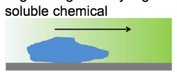
Heterogeneous milieu problem: how do we integrate information about spatial and biological heterogeneity of the wound?

Statistics of directed migration from chemotaxis and haptotaxis experiments

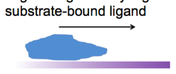
Microfluidics chamber scheme

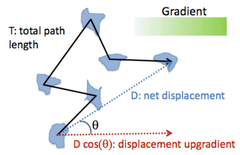


Chemotaxis: Migration guided by a gradient of soluble chemical

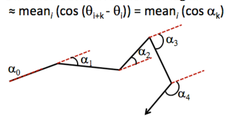


Haptotaxis: Migration guided by a gradient of substrate-bound ligand

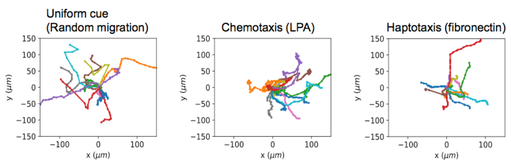




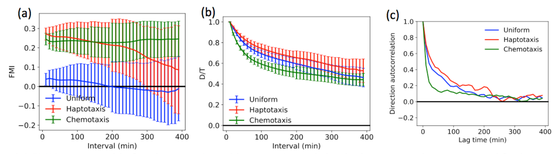
Direction autocorrelation for lag k:
≈ mean$_i$ (cos (θ$_{i+k}$ - θ$_i$)) = mean$_i$ (cos α$_k$)



Sample tracks

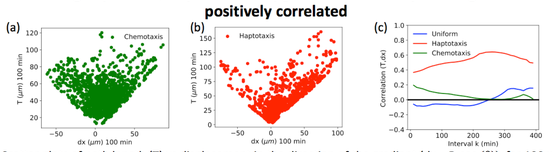


Chemotaxing cells are less persistent than haptotaxing cells



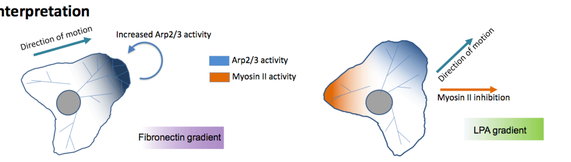
(a) FMI is comparable for chemotaxis and haptotaxis, and slowly decreases when computed for larger intervals in haptotaxis. Persistence over a period of a few hours is reduced for chemotaxis, as measured by D/T (b) and direction autocorrelation (c).

During haptotaxis, displacement upgradient and total path length are positively correlated



Scatter plots of path length (T) vs displacement in the direction of the gradient (dx = D cos (θ)) for 100min intervals during chemotaxis (a) and (b) haptotaxis. (c) Correlation coefficient between T and dx for different time intervals.

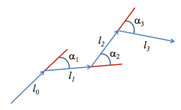
Interpretation



As a haptotaxing cell encounters higher concentration of fibronectin, Arp2/3 activity at the leading edge increases, fostering protrusion and motility.

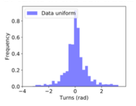
Directional bias in Myosin II activity interferes with the direction of motion of the cell which is dictated by lamellipodia orientation and Arp2/3 signaling. As a consequence, persistence is reduced.

Persistent walk simulations: memory from the previous step

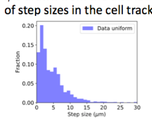


$\theta_i = \theta_{i-1} + \alpha_i$

α_i is drawn from the distribution of turns in the cell tracks



l_i is drawn from the distribution of step sizes in the cell tracks

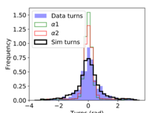


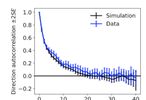
Persistent walk simulations with 2-processes

1. Internal orientation
$\omega_i = \omega_{i-1} + \alpha 1_i$

2. Cell direction of motion
$\theta_i = \{ (1\text{-}w)\theta_{i-1} + w\,\omega_i \} + \alpha 2_i$

α1, α2 : Drawn from a mixed von Misses - uniform distribution
w: Weight factor in [0,1]





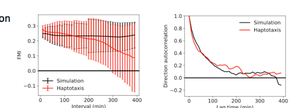
Two-processes model fits direction autocorrelation data

Biased 2-processes walk simulation

$\omega_i = \{ (1\text{-}tx)\,\omega_{i-1} + tx\,\gamma_{eff} \} + \alpha 1$

$\theta_i = \{ (1\text{-}w)\theta_{i-1} + w\,\omega_i \} + \alpha 2$

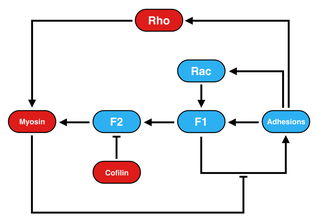
$\gamma_{eff} = \gamma + \varepsilon$ detected gradient direction
γ: Direction of the gradient
ε: Noise in gradient detection
tx: bias factor in [0,1]

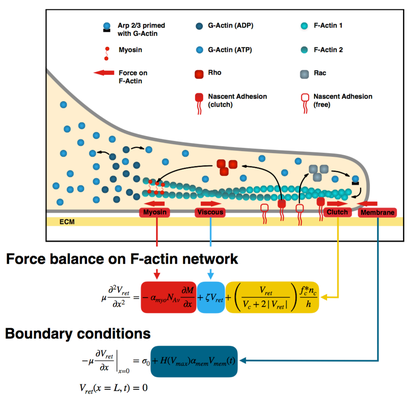


Integrating adhesion, signaling, and actin dynamics

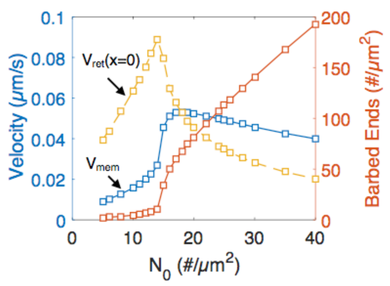
Our physicochemical model combines adhesion and dendritic actin dynamics. Nascent adhesions affect the F-actin network by mediating activation of Rho-family GTPases and mechanically resisting retrograde flow. Rho/ROCK signaling enhances myosin II motor activity, which is also affected by the PLC/PKC pathway during fibroblast chemotaxis.

Model	Signaling	Explicit Actin Dynamics	Explicit Adhesion Dynamics	Explicit Mechanics /Force Balance	Spatial	Other
Current Model	+	+	+	+	+	
Nickaeen et al., PLoS Comput. Biol. (2017).	-	-	-	+	+	2D, moving boundary
Aroush et al., Curr. Biol. (2017).	-	+	-	+/-	+	
Holmes et al., PLoS Comput Biol (2017).	+	-	-	-	-	
Barnhart et al., Curr. Biol. (2017).	-	-	+	-	+	
Copos et al., Biophys. J. (2017).	-	-	+	+	+	Whole cell model
Cheng et al., Biophys. J. (2016).	+/-	+/-	+	+	-	Filopodial dynamics
Craig et al., Phys. Biol. (2015)	-	-	+	+	+	
Welf et al., Mol. Biol. Cell. (2013)	+/-	-	+	+	-	
Tani et al., Biophys. J. (2013).	-	+/-	-	-	+	
Hu & Papoian, J. Phys. Chem. (2013).	-	+	-	+	+	3D
Shemesh et al., Biophys. J. (2012).	-	-	+	+	+	2D
Craig et al., Biophys. J. (2012).	-	+	-	+	+	
Walcott & Sun, Proc. Natl. Acad. Sci. (2010).	-	-	+	+/-	+	2D
Chan & Odde, Science. (2008).	-	-	+	-	+	
Gracheva & Othmer, Bull. Math. Biol. (2004).	+/-	-	+	+	+	Includes cell rear
Mogilner & Edelstein-Keshet, Biophys. J. (2002).	-	+	-	+/-	+	

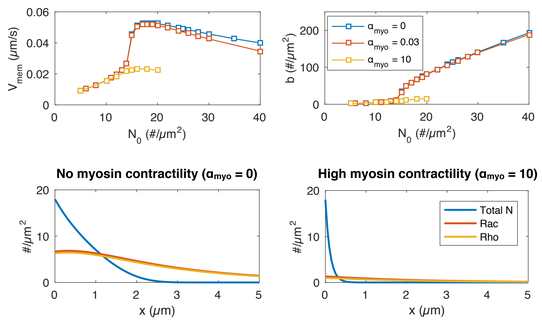




Adhesion-F-actin feedback promotes protrusion



Myosin II mechanically disrupts pro-protrusion feedback

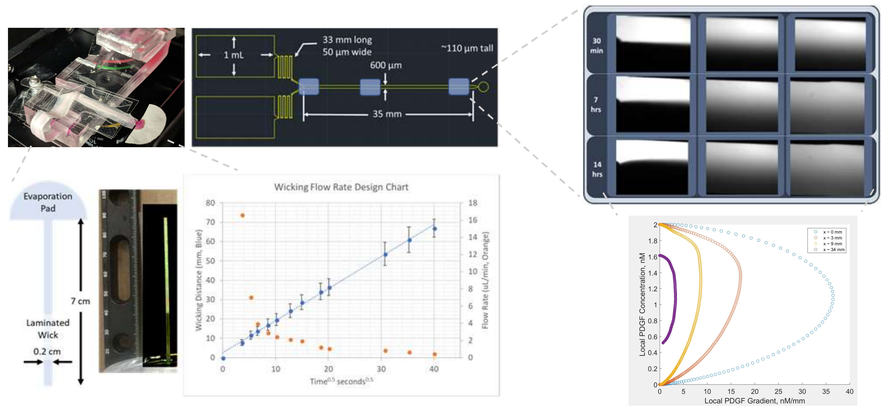


G-actin fuels protrusion; F-actin turnover enhances G-actin flux and reduces actomyosin

Not shown: Can mechanical compliance of the adhesion/F-actin linkage explain durotaxis?

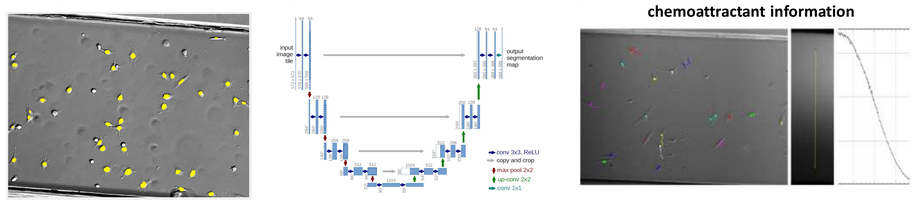
A new experimental and analysis workflow

Modified Y-junction design generates stable, tunable gradients



U-net deep learning automates segmentation, enabling high-throughput cell tracking (collaboration with Dr. Kevin Flores, NCSU)

Label ground truth nuclei *in situ* | **Segment nuclei with U-net [3] deep learning** | **Automatedly build trajectories with local chemoattractant information**



[3] Ronneberger, Fischer, and Brox. *Int Conf Med img comp & comp-asst intervention.* (2015).

Model credibility

All of our models are formulated with the intent to publish the work in peer-reviewed journals. In publications, care is taken to explain:

- The context for which each model is used, including the biological significance;
- The model's variables, parameters, processes, and structure(s), with citation of associated literature;
- Explicit and underlying model assumptions and associated justifications;
- Numerical testing of the model according to accepted standards;
- Important limitations of the model.

Together with the provision of the models in executable form (e.g., source code), annotated according to accepted standards, these steps ensure that our modeling results are repeatable and reproducible, and that our models may be readily adaptable by others. We are keen to discuss ways that we might improve our internal workflow, including version control and electronic notebooks.